

a1 1992; Egholm et al., *Nature* 365:566-568, 1993; Almarsson et al., *Proc. Natl. Acad. Sci. U. S. A.* 90:9542-9546, 1993; Demidov et al., *Proc. Natl. Acad. Sci. U. S. A.* 92:2637-2641, 1995). They have also been shown to be resistant to nuclease and protease digestion (Demidov et al., *Biochem. Pharm.* 48:1310-1313, 1994). DNA analogs such as phosphorothioates are also contemplated herein (see U.S. Patent No. 5,459,127).

IN THE CLAIMS

Please cancel claim 4.

Kindly amend claim 3 as follows:

a2 3. (Amended) The method of claim 1, wherein said promoter-containing sequence and said terminator-containing sequence further comprise a PNA binding domain.

[Kindly add new claims 5-45 as set forth below:]

a3 5. (New) The method of claim 2, wherein said polymerase is a non blunt end polymerase.

6. (New) The method of claim 3, wherein the non blunt end polymerase is Taq polymerase.

7. (New) The method of claim 1, wherein said PCR-amplifying comprises the addition of binding moiety.

8. (New) The method of claim 7, wherein said binding moiety comprises a PNA molecule.

9. (New) The method of claim 7, wherein said binding moiety comprises at least one phosphorothioate.

10. (New) A method of adding a nucleic acid sequence that confers function to a polynucleotide target sequence, comprising:

contacting a first nucleic acid fragment with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and an extension region;

PCR amplifying the first nucleic acid fragment and the polynucleotide target sequence, to form a second nucleic acid fragment that comprises the polynucleotide target sequence and the extension region;

contacting the second nucleic acid fragment with a third nucleic acid fragment that comprises a region complementary to the extension region and also a nucleic acid sequence that confers function; and

PCR amplifying the second nucleic acid fragment with the third nucleic acid fragment to form a fourth nucleic acid fragment that comprises the nucleic acid sequence that confers function joined to the polynucleotide target sequence.

11. (New) The method of claim 10, wherein said region complementary to the extension region is not specifically complementary to a region on said polynucleotide target sequence.

12. (New) The method of claim 10, wherein the nucleic acid region that confers function comprises a promoter or a terminator.

13. (New) The method of claim 10, wherein the PCR amplifying step is accomplished using a polymerase.

14. (New) The method of claim 13, wherein the polymerase is a non blunt end polymerase.

15. (New) The method of claim 14, wherein the non blunt end polymerase is Taq polymerase.

16. (New) The method of claim 10, wherein said nucleic acid sequence that confers function and said second nucleic acid fragment include an internal nucleotide capable of forming an A-T base pair immediately adjacent to said extension region.

17. (New) The method of claim 10, wherein said step of PCR amplifying of the second nucleic acid fragment with the third nucleic acid fragment further comprises a primer, wherein said primer comprises a nuclease resistant binding moiety that upon amplification confers nuclease resistance to said fourth nucleic acid fragment.

18. (New) The method of claim 17, wherein said nuclease resistant binding moiety comprises a PNA molecule.

19. (New) The method of claim 17, wherein said nuclease resistant binding moiety comprises at least one phosphorothioate.

20. (New) The method of claim 10, wherein third nucleic acid fragment further comprises a PNA binding domain.

21. (New) A method of adding a nucleic acid sequence that confers function to a polynucleotide target sequence, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

PCR amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an intermediate nucleic acid fragment that comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with third and fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions, wherein one or both of the third and fourth fragments further comprise a nucleic acid region that confers function; and

PCR amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments to form a product nucleic acid fragment that comprises one or more functional nucleic acid regions joined to the polynucleotide target sequence.

22. (New) The method of claim 21, wherein the product nucleic acid fragment comprise a promoter sequence or a terminator sequence or both.

23. (New) The method of claim 21, wherein said functional nucleic acid fragment further comprises a PNA binding domain.

24. (New) The method of claim 21, wherein said PCR amplifying of the intermediate nucleic acid fragment further comprises a set of primers comprising one or more nuclease resistant, binding moieties that upon amplification confers nuclease resistance to said product nucleic acid fragment.

25. (New) The method of claim 24, wherein said one or more nuclease resistant, binding moieties comprise a PNA molecule or one or more phosphorothioate molecules or both.

26. (New) The method of claim 21, wherein said PCR amplifying steps are accomplished using a polymerase.

27. (New) The method of claim 26, wherein said polymerase is a non blunt end polymerase.

28. (New) The method of claim 27, wherein said non blunt end polymerase is Taq polymerase.

29. (New) The method of claim 21, wherein said nucleic acid region that confers function comprises a promoter or a terminator.

30. (New) A system for adding a nucleic acid fragment that confers function to a polynucleotide target sequence, comprising:

an extension primer pair, each primer of which comprises a region of complementarity to a strand of the polynucleotide target sequence and a predetermined extension region; and

a pair of biological function conferring nucleic acid fragments, each fragment of which comprises a region of complementarity to one of the extension regions, and a biological function conferring polynucleotide sequence that confers biological function, wherein the extension primer pairs are adapted to add the extension regions to a target sequence upon a first PCR procedure, and the function conferring nucleic acid pairs are adapted to add the functional polynucleotide sequences to the polynucleotide target sequence upon a second PCR procedure.

31. (New) The system of claim 30, wherein the system further comprises a polymerase.

32. (New) The system of claim 31, wherein the polymerase is a non blunt end polymerase

33. (New) The system of claim 32, wherein the non blunt end polymerase is Taq polymerase.

34. (New) The system of claim 30, wherein the biological function conferring nucleic acid fragments comprise a promoter and/or a terminator.

35. (New) The system of claim 34, wherein the biological function conferring nucleic acid fragments further comprise at least one PNA binding domain.

36. (New) The system of claim 30, further comprising an additional primer pair comprising at least one nuclease-resistant, binding moiety.

37. (New) The system of claim 36, wherein the nuclease-resistant, binding moiety is selected from the group consisting of a PNA molecule and a phosphorothioate.

38. (New) A method for creating transcriptionally-active nucleic acid sequences from a plurality of different target polypeptide-encoding sequences, comprising:

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creating extension primer pairs for each target sequence, each extension primer pair comprising first and second extension primers, respectively comprising first and second extension regions and a region of complementarity to a particular target sequence, such that the first and second extension regions for each extension primer pair are the same as the first and second extension regions for the other of said extension primer pairs, but the regions of complementarity are customized for each target sequence;

PCR-amplifying each of said target sequences with said extension primer pairs to provide intermediate sequences comprising said plurality of target sequences, each said target sequence flanked by the same first and second extension regions; and

providing transcriptionally-functional fragment pairs, wherein each transcriptionally-functional fragment pair comprises a first fragment having a region of complementarity to the first extension region and a second primer having a region of complementarity to the second extension region, and at least one of said fragments in said transcriptionally-functional fragment pair comprising a transcriptionally-functional region; and

PCR-amplifying each of said intermediate sequences with said transcriptionally-functional fragment pairs to provide a plurality of transcriptionally-functional polynucleotides, each comprising one of the target sequences linked to at least one transcriptionally-functional region.

39. (New) The method of claim 38, wherein said transcriptionally-functional region is a promoter or a terminator sequence.

40. (New) The method of claim 38, wherein said transcriptionally-functional fragment pair adds both a promoter and a terminator to the target sequence.

41. (New) The method of claim 38, wherein the PCR amplifications are performed separately for each of said target sequences.

42. (New) The method of claim 38, wherein said PCR-amplifying steps are accomplished using a polymerase.

43. (New) The method of claim 42, wherein said polymerase is a non blunt end polymerase.

44. (New) A method of generating a nuclease resistant and functional nucleic acid molecule, comprising:

contacting first and second nucleic acid fragments with a polynucleotide target sequence, wherein the first nucleic acid fragment comprises a first region of complementarity to a portion of the polynucleotide target sequence and a first extension region, and the second nucleic acid fragment comprises a second region of complementarity to a second portion of the polynucleotide target sequence and a second extension region;

PCR amplifying the first and second nucleic acid fragments and the polynucleotide target sequence, to form an intermediate nucleic acid fragment that comprises the polynucleotide target sequence flanked by the first and second extension regions;

contacting the intermediate nucleic acid fragment with a third and a fourth nucleic acid fragments that respectively comprise a region complementary to the first and second extension regions and with a first primer and a second primer at least one of which comprises a nuclease-resistant, binding moiety, wherein one or both of the third and forth nucleic acid fragments further comprise a nucleic acid region that confers function; and

PCR amplifying the intermediate nucleic acid fragment with the third and fourth nucleic acid fragments and the first and second primers to form a nuclease resistant nucleic acid molecule that comprises one or more functional nucleic acid regions joined